Express Mail No. **EV 346 811 873 US**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Nurit LIVNAH et al.

Confirmation No. 9174

Application No.: 10/764,288

Group Art Unit: 1614

Filing Date: January 23, 2004

Examiner:

Atty. Docket No.: 87534-4300

For: PROTEIN KINASE INHIBITORS COMPRISING ATP MIMETICS CONJUGATED TO PEPTIDES OR PEPTIDOMIMETICS

SUBMISSION OF CERTIFIED PRIORITY DOCUMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

Applicants have claimed priority of Israeli application no. IL144583 filed July 26, 2001, under 35 U.S.C. § 119. In support of this claim, a certified copy of said application is submitted herewith.

No fee or certification is believed to be due for this submission. Should any fees be required, however, please charge such fees to Winston & Strawn LLP Deposit Account No. 50-1814.

Respectfully submitted,

Date: 10/8/04

Allan A. Fanucci (Reg. No. 30,256)
WINSTON & STRAWN LLP
CUSTOMER NO. 28765
(212) 294-3311

Enclosures

NY:901045.1

חוק הפטנטים, תשכ"ז - 1967
PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

לשימוש הלשכה
For Office Use

מספר: 44583
Number

תאריך: 7- 2001
Date
הוקדם/נדחה
Ante/Post-dated

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
(Name and address of applicant, and in case of body corporate-place of incorporation)

פפטור בע"מ, חברה ישראלית
קריית וייצמן רחובות 76326

P[illegible] LTD,
K[illegible]eizmann, Rehovot 76326

הממציאים: נורית ליבנה, יוסף סליטרה, תמר יחזקאל
Th[illegible]ors: Nurit Livnah, Yoseph Salitra, Tamar Yechezkel

בעל אמצאה מכח החוק
Owner, by virtue of The law

of [illegible]he title of which is

(בעברית) **מעכבי קינזות כימריות של חלבונים**
(Hebrew)

(באנגלית) CHIMERIC PROTEIN KINASE INHIBITORS
(English)

מבקש בזאת כי ינתן לי עליה פטנט
hereb[illegible]tent to be granted to me in respect thereof.

*בקשת פטנט מוסף - Application for Patent Addition	*דרישה דין קדימה Priority Claim		
*לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
מס'.....No..... מיום.....dated.....			
* יפוי כח: כללי / ~~מיוחד - רצוף בזה~~ ~~attached / to be filed later~~ -			
הוגש בעניין.....5096.....			

המען למסירת מסמכים בישראל
Address for Service in Israel

P/02 מס[illegible]

C[illegible]

ד"ר ס. וב[illegible]
[illegible] & Associates
ת.ד. [illegible]9
רחובות

העתק מאושר
ממונה על הבוחנים
ירושלים 24/7/01

חתימת המבקש
[illegible]gnature of Applicant

Dr. C[illegible]
Patent[illegible]ney

היום ...24..... בחודש .....יולי..... שנת .2001..
This of the year

לשימוש הלשכה
For Office Use

טופס זה כשהוא מוטבע בחותם לשכת הפטנטים ומושלם במספר ובתאריך ההגשה, הנו אישור להגשת הבקשה שפרטיה רשומים לעיל.
This form impressed with the Seal of the Patent Office and indicating the number and date of filling, certifies the filing of the

CHIMERIC PROTEIN KINASE INHIBITORS

מעכבי קינזות כימריות של חלבונים

P/021

CHIMERIC PROTEIN KINASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to isoquinoline derivative conjugates, to pharmaceutical compositions containing the isoquinoline derivatives and conjugates, their use as inhibitors of protein kinase, as well as to processes for the preparation and use of such molecules.

BACKGROUND OF THE INVENTION

Protein kinases are involved in signal transduction pathways linking growth factors, hormones and other cell regulation molecules to cell growth, survival and metabolism under both normal and pathological conditions. The superfamily of protein kinases includes protein kinase A and protein kinase C, as well as the more recently discovered protein kinase B (PKB). PKB is a direct downstream effector of phosphatidylinositol 3-kinase (PI 3-kinase) and is activated in response to insulin or growth factors (for review see Kandel and Hay, Exp. Cell Research 253, 210, 1999).

PKB activation involves phosphorylation of two amino acid residues, Ser473 and Thr308. PKB is a newly recognized anti-apoptotic protein kinase whose activity is strongly elevated in human malignancies. PKB was originally discovered as a viral oncogene v-Akt in rat T-cell leukemia. It was later established that v-Akt is the oncogenic version of a cellular enzyme PKB/c-Akt, in which a truncated viral group specific antigen, gag, is fused in frame to the full length Akt-1 and is membrane bound whereas PKB/c-Akt is cytoplasmic. Sequencing of Akt revealed a high degree of homology to PKA (~75%) and PKC isozymes (~50%), a fact which lent its rechristening as PKB.

The enzyme is activated by the second messenger PIP3 produced by PI'-3-kinase. PIP3 binds to the pleckstrin homology (PH) domains of PKB, recruits it to the membrane where it is phosphorylated and converted to its activated form. Since PKB activation is PI'-3-kinase dependent, the persistent activation of certain protein tyrosine kinases, such as IGF-1 receptor, EGF receptor, PDGF receptor, pp60c-Src, and the like, leads to the persistent activation of PKB which is indeed encountered in many tumors. Deletions in the gene coding for the tumor suppressor PTEN also induces the persistent activation of

PKB/cAkt since it is the negative regulator of this enzyme . Also, PKB is overexpressed in 15% of ovarian cancers, 12% of pancreatic cancers and 3% of breast cancers, and was shown to produce a survival signal that protects cells from apoptosis thus contributing to resistance to chemotherapy.

These molecular properties of PKB and its central role in tumorigenesis, implies that this protein kinase may be an attractive target for novel anti-cancer agents. To date no specific inhibitors of PKB are known in the art, nor are any of the disclosed inhibitors of protein kinases A and C known to act on PKB.

Hidaka H. et al. (Biochemistry, 32, 5036, 1984) describe a class of isoquinolinesulfonamides having inhibitory activity towards cyclic nucleotide dependent protein kinases (PKA and PKG) and protein kinases C (PKC). The same class of compounds is claimed in EP 061673, which discloses said compounds as having cardiovascular activity. Additional derivatives of isoquinolinesulfonyl were disclosed by Hidaka in EP 109023, US 4456757, US 4525589, and US 4560755.

Antitumor activity has been suggested for some of these isoquinolinesulfonamides. Martell R.E. et al. (Biochem. Pharm., 37, 635, 1988) found effects of two isoquinolinesulfonamides, namely 1-(5-isoquinolinsulfonyl) -2-methylpiperazine (H-7) and N-[2-guanidinoethyl]- 5-isoquinolinesulfonamide (HA-1004), which have a certain selectivity for PKC and cyclic nucleotide dependent protein kinases, respectively, on calcitriol-induced cell differentiation. Further, Nishikawa M. et al., Life Sci., 39, 1101, 1986), demonstrate that the same compound H-7 inhibits cell differentiation induced by phorbol diester.

International PCT application WO 93/13072 discloses 5-isoquinolinesulfonamide derivatives as protein kinase inhibiting agents wherein the claimed compounds all contain two sulfonyl moieties.

Other classes of compounds known in the prior art (EP-A-397060, DE-A-3914764 and EP-A-384349) showed the capacity of inhibiting protein kinases, however, said compounds have a chemical structure which is totally different from that of the compounds of the present invention. In addition, international PCT application WO 98/53050 discloses short peptides derived from the HJ loop of a serine/threonine kinase which modulate the activity of serine/threonine kinases.

The minimal consensus sequence for efficient phosphorylation by PKB was found by Alessi et al. (Fed. Eur. Biochem. Soc., 399, 333, 1996). This is a 7-mer motif with the most active peptide substrate having the sequence Arg-Pro-Arg-Thr-Ser-Ser-Phe.

International application WO 97/22360 discloses certain PKB substrate peptides having 7-amino acids length, useful as substrate for measuring PKB activity.

Obata et al. (J. Biol. Chem., 17, 36108, 2000) described the use of an oriented peptide library approach to determine optimal amino acid sequence of the PKB substrate. All the substrates identified contained the known motif having the sequence Arg-Xaa-Arg-Xaa-Saa-Ser/Thr.

Parang et al. (Nature Structural Biology 8, 37, 2001), describe peptide-ATP bisubstrate analogs of a protein kinase A inhibitor, wherein ATP is linked to a protein kinase peptide substrate. Nevertheless, this approach has a limitation of suboptimal pharmacokinetic properties.

The present invention overcomes these limitations by providing ATP surrogates and peptidomimetics with protein target specificity.

Disclosures in the background art and in Israeli application No. 136458 relate to numerous specific isoquinoline derivatives, which are PKB inhibitors. The present invention is directed to novel isoquinoline derivatives and more specifically isoquinoline conjugates, and excludes all of the known compounds previously claimed for their capacity to inhibit PKB.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide small molecule inhibitors of protein kinases for medical, therapeutic and drug design purposes. It is yet another object of the present invention to provide such molecules, which are selective inhibitors of protein kinase B.

One aspect of the present invention involves the preparation of novel compounds which inhibit the activity of protein kinases. It has now surprisingly been found that certain novel derivatives of isoquinolinesulfonamides, which are protein kinase inhibiting agents, proved to be selectively active towards a specific type of protein kinase, namely protein kinase B.

Another aspect of the present invention is directed to pharmaceutical compositions comprising as an active ingredient inhibitors of protein kinase and to methods for the preparation of pharmaceutical compositions comprising inhibitors of protein kinases.

Another aspect of the present invention is directed to the use of pharmaceutical compositions comprising these protein kinase inhibitors for production of medicaments useful for the treatment or diagnosis of diseases and disorders. The present invention discloses methods of treatment of disorders wherein protein kinase is involved including but not limited to cancers, cardiovascular pathologies, hemorrhagic shock, obesity, inflammatory diseases, diseases of the central nervous system, and autoimmune diseases.

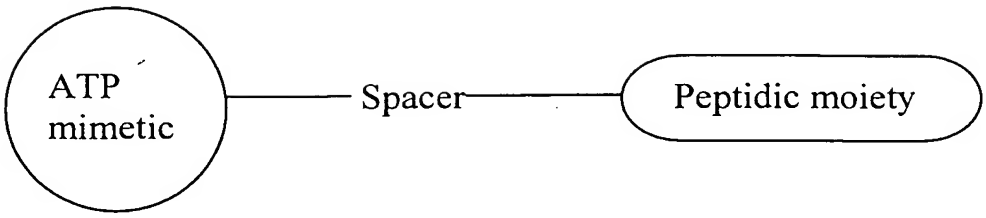
It is another object of the present invention to provide methods for modulating the activity of protein kinases in a subject, comprising administering a therapeutically effective amount of protein kinase inhibitors.

Further objects of the present invention are directed to methods for the diagnosis of diseases including in-vitro diagnosis using the compounds of the present invention, and in-vivo diagnosis involving administering a pharmaceutical composition comprising a diagnostically useful amount of a protein kinase inhibitor prepared according to the principles of the present invention.

It is yet another object of the present invention to provide small molecules that mimic the ATP molecule of the PKB which are conjugated to a peptide substrate or peptido-mimetic substrate of PKB. The chimeric compounds according to the present invention preferably serve as PKB inhibitors with improved activity and selectivity.

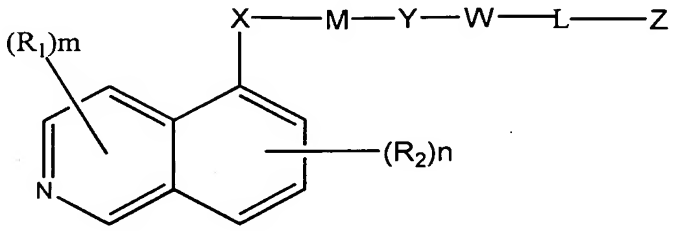
Yet, it is another object of the present invention to provide peptidomimetic compounds having improved stability and cell permeability properties. Non limiting examples of generation of such compounds include N-alkylation of selected peptide residues, use of carbamate, urea and hydrazine as peptide bond replacement, and incorporation of hetrocyclic non-peptide moieties such as piperidine, piperazine, pyrrolidine etc. through peptide or non-peptide bond. These peptidomimetic compounds may be used according to the present invention as the peptide substrate part of chimeric compounds. In addition these peptidomimetic compounds may be used as protein kinase inhibitors per se.

Preferred embodiments according to the present invention comprise a chimeric compound comprising both an ATP mimetic moiety and a peptidic substrate, as described in the following scheme:



The ATP mimetic core includes but is not limited to dansyls, isoquinolines, quinolines and naphthalenes. The spacer is of varied lengths and conformations of any suitable chemistry including but not limited to amine, amide, thioether, oxyether, sulfonamide bond and the like. Non limiting examples for such spacers include sulfone amide derivatives, amino thiol derivatives and amino alcohol derivatives. The peptidic moiety comprises peptides and peptidomimetics. Such inhibitory peptides may be designed based on any peptide which may serve as PKB substrate.

Additional more preferred embodiment of the present invention comprises a compound of Formula I



Formula I

wherein:

R_1 and R_2 are independently selected from the group consisting of hydrogen, a lower alkyl group, a lower alkoxy group, substituted or unsubstituted phenyl group, a lower alkyl substituted with at least one substituent selected from the group consisting of a phenyl group, a halogen, hydroxyl, thiol, nitro, cyano, or amino group;

m and n are each independently 0-3;

X is selected from the group consisting of SO_2-NH, S and O;

M represents substituted or unsubstituted alkylene of 1-4 carbon atoms;

Y is selected from the group consisting of amide, amine, urea, carbamate, hydrazine or sulfonamide;

W is absent or is selected form the group consisting of substituted or unsubstituted alkylene, aliphatic, aromatic or heterocyclic moiety, of 1-18 carbon atoms;
L is absent or is selected from the group consisting of amide, amine, urea, carbamate, hydrazine or sulfonamide; and
Z is a peptide or peptidomimetic moiety of 4-12 residues in length capable of binding to the substrate site of PKB.

Preferred peptide substrate and peptide substrate mimetics according to the present invention, forming part of the chimeric compounds are described in the following scheme:

AA_1—— AA_2—— AA_3—— AA_4—— AA_5—— AA_6——AA_7

According to this scheme AA is selected from the group consisting of an amino acid, an amino acid analog, or an aliphatic, aromatic or heterocyclic moiety, incorporated into the sequence to create a peptidomimetic moiety with improved pharmacological properties.

AA_1 and AA_3 are independently selected from the group consisting of: arginine or arginine analog; lysine or lysine analog; ornithine or ornithine analog; or an aliphatic, aromatic, or heterocyclic moiety bearing a group positively charged at physiological pH, such as an amine, guanidine or amidine, homoarginine, argininol.

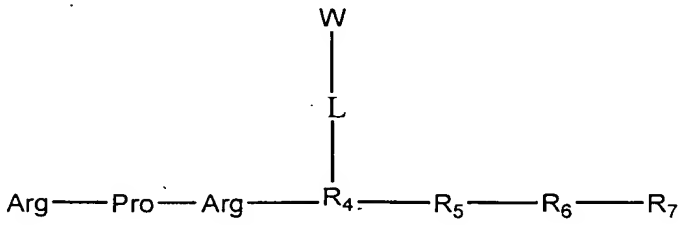
AA_2 is selected from the group consisting of proline, proline analog or an aliphatic, aromatic or heterocyclic moiety, hydroxyproline, nipecotic acid, alanine, aminobutyric acid.

AA_4, AA_5, AA_6 are independently selected from the group consisting of: Thr or Thr analog; Ser or Ser analog; Ala or Ala analog; Glu or Glu analog; an aliphatic, aromatic or heterocyclic residue bearing alkyl, benzyl, hydroxy, phenoxy alkoxy, sulfone, sulfoxide, phosphonate, phosphonate ester, amide or carbamoyl functionality, amino butyric acid, citrulline, serinol, phosphotyrosine and phosphotyrosine dimethyl ester.

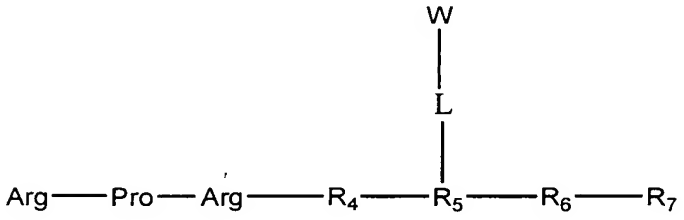
AA_7 is selected from the group consisting of: Phe or Phe analog; Trp, Tyr, Leu, Ile, and their analogs; aromatic moiety esters or aromatic substitutions of an amino acid; an aromatic, heterocyclic or branched aliphatic moiety; homophenylalanine, homoleucine, glutamic benzyl ester, naphtylalanine.

Due to the peptidomimetic nature of the preferred embodiments according to the invention, the bonds between AAs are not only peptide bonds but may be selected from the group consisting of: an amide, urea, carbamate, hydrazine or sulfoneamide bond.

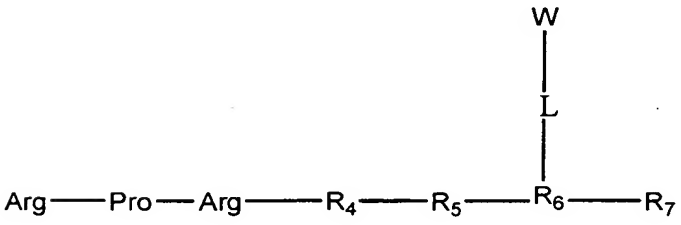
Additional preferred embodiments of the present invention comprise a compound of Formulae IIa - IId:



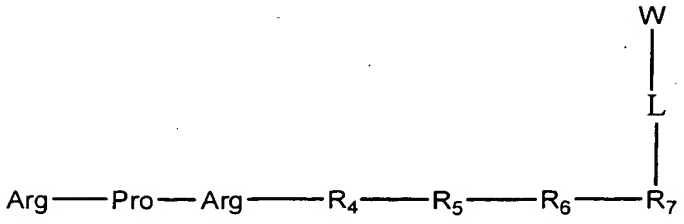
Formula IIa



Formula IIb



Formula IIc



Formula IId

wherein:

R_4 is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R_5 and R_6 are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

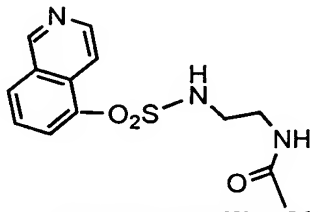
R_7 is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R_7 represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid.

One currently most preferred embodiment of the present invention is the chimeric compound denoted hereinbelow as PTR 6016 described in Formula III:



Arg-Pro-Arg-Thr-Glu-Ser-Phe

Formula III

Essentially all of the uses known or envisioned in the prior art for protein kinase inhibitors, can be accomplished with the molecules of the present invention. These uses include therapy and diagnostic techniques.

By way of exemplification, the compounds disclosed in the present invention were selected for inhibition of Protein kinase B. Using the preparations and methods disclosed herein it is possible to obtain compounds which inhibit the activity of other types of protein kinases.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. The synergistic effect of chimeric compound PTR 6016 composed of an ATP mimic and a peptide substrate.

Figure 2. Inhibition activity curve for AR-60003-52 as described in example 7.

DETAILED DESCRIPTION OF THE INVENTION

It is now disclosed that small molecules according to the present invention are inhibitors of protein kinases. In general, it has now been discovered that the active molecules according to the present invention share certain structural motifs, which may be construed as an "ATP mimetic" motif. This motif, in the broadest terms may be defined as comprising four distinct regions, which are defined by their functional and chemical attributes, as will be exemplified hereinbelow.

The disclosed protein kinase inhibitors are small molecules which exhibit enhanced specificity toward certain protein kinase subtypes. In principle, the present invention provides for the first time selective inhibitors of protein kinase B. The preferred molecules generally have a molecular weight of less than about 1000 daltons. These and further advantages over the background art will become apparent from the description of the currently preferred embodiments of the present invention.

Additional more preferred compounds are composed of small molecules mimicking the ATP molecule of the PKB, which are conjugated to a peptide substrate or peptido-mimetic substrate of PKB. The chimeric compounds according to the present invention preferably serve as PKB inhibitors with improved activity and selectivity.

The utility of the compositions according to the invention can be established by means of various assays as are well known in the art. The preferred compounds of the

present invention were found to be active in a panel of in-vitro assays, in inhibiting the activity of protein kinases and in induction of apoptosis in cancer cells.

Pharmaceutical compositions according to the present invention comprising pharmacologically active protein kinase inhibitors and a pharmaceutically acceptable carrier or diluent represent another embodiment of the invention, as do the methods for the treatment of a mammal in need thereof with a pharmaceutical composition comprising an effective amount of a protein kinase inhibitor according to the invention. Methods of treatment using the compositions of the invention are useful for therapy of cancers, diabetes, cardiovascular pathologies, hemorrhagic shock, obesity, inflammatory diseases, diseases of the central nervous system, and autoimmune diseases using such compositions. The pharmaceutical compositions according to the present invention advantageously comprise at least one protein kinase inhibitor. These pharmaceutical compositions may be administered by any suitable route of administration, including topically or systemically. Preferred modes of administration include but are not limited to parenteral routes such as intravenous and intramuscular injections, as well as via nasal or oral ingestion.

In the specification and in the claims the term "therapeutically effective amount" refers to the amount of protein kinase inhibitor or composition comprising same to administer to a host to achieve the desired results for the indications described herein, such as but not limited of cancers, diabetes, cardiovascular pathologies, hemorrhagic shock, obesity, inflammatory diseases, diseases of the central nervous system, and autoimmune diseases.

In the specification and in the claims the term "protein kinase" refers to a member of an enzyme superfamily which functions to phosphorylate one or more protein as described above.

As used herein and in the claims, the term "inhibitor" is interchangeably used to denote "antagonist" these terms define compositions which have the capability of decreasing certain enzyme activity or competing with the activity or function of a substrate of said enzyme.

As used herein and in the claims the term "chimeric compound" or "chimeric molecule" denotes an ATP mimic moiety conjugated to a PKB substrate part. Examples for such chimeric compounds or conjugates are small molecules (and more specific isoquinoline derivatives) that mimic the ATP molecule of the PKB, conjugated to a peptide substrate or peptido-mimetic substrate of PKB. These molecules may preferably serve as PKB inhibitors with improved activity and selectivity.

Certain abbreviations are used herein to describe this invention and the manner of making and using it. For instance, ATP refers to adenosine three phosphate, BSA refers to bovine serum albumin, BTC refers to bis-(trichloromethyl)carbonate or triphosgene, DCM refers to dichloromethane, DIEA refers to diisopropyl-ethyl amine, DMF refers to dimethyl formamide, EDT refers to ethanedithiol, EDTA refers to ethylene diamine tetra acetate, ELISA refers to enzyme linked immuno sorbent assay, EGF refers to epithelial growth factor, FACS refers to fluorescence assisted cell sorter, HA refers to hemagglutinin, HBTU refers to 1-hydroxybenztriazolyltetramethyl-uronium, HEPES refers to 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, HOBT refers to 1-hydroxybenzotriazole, HRP refers to horse raddish peroxidase, IGF refers to insulin growth factor , MOPS refers to 4-morpholinepropanesulfonic acid, MPS refers to multiple parallel synthesis, NMP refers to N-methyl formamide, OPD refers to o-Phenylenediamine, PBS refers to phosphate buffer saline, PKA refers to protein kinase A, PKB refers to protein kinase B, PKC refers to protein kinase C, rpm refers to rounds per minute, SAR refers to structure-activity relationship, THF refers to tetrahydrofuran, TIS refers to tri-isopropyl-silane, TFA refers to trifluoric acetic acid.

As used herein "peptide" indicates a sequence of amino acids linked by peptide bonds. The peptide analogs of this invention comprise a sequence of 3 to 15 amino acid residues, preferably 4 to 12 residues, more preferably 5 to 10 amino acids, each residue being characterized by having an amino and a carboxy terminus.

The term "peptidomimetic" means that a peptide according to the invention is modified in such a way that it includes at least one non-coded residue or non-peptidic bond. Such modifications include, e.g., alkylation and more specific methylation of one or more residues, insertion of or replacement of natural amino acid by non-natural amino acids, replacement of an amide bond with other covalent bond. A peptidomimetic

according to the present invention may optionally comprises at least one bond which amide-replacement bond such as urea bond, carbamate bond, sulfonamide bond, hydr bond, or any other covalent bond. The design of appropriate "peptidomimetic" may b computer assisted.

The term "peptide analog" indicates molecule which has the amino acid sequence according to the invention except for one or more amino acid changes.

The amino acids used in this invention are those which are available commerci or are available by routine synthetic methods. Certain residues may require special methods for incorporation into the peptide, and either sequential, divergent and conve synthetic approaches to the peptide sequence are useful in this invention. Natural code amino acids and their derivatives are represented by three-letter codes according to IU conventions. When there is no indication, the L isomer was used. The D isomers are indicated by "D" before the residue abbreviation.

Conservative substitution of amino acids as known to those skilled in the art ar within the scope of the present invention. Conservative amino acid substitutions includ replacement of one amino acid with another having the same type of functional group side chain e.g. aliphatic, aromatic, positively charged, negatively charged.

List of non limiting examples of non-coded amino acids which where used in t present invention: Abu refers to 2-aminobutyric acid, ArgOl refers to argininol, Bpa re to 4-Benzoylphenylalanine, Bip refers to Beta-(4-biphenyl)-alanine, Dap refers to Diaminopropionic acid, Dim refers to Dimethoxyphenylalanine, Dpr refers to Diaminopropionic acid, Hol refers to homoleucine, HPhe refers to Homophenylalanine GlyNH2 refers to Aminoglycine, Nle refers to Norleucine, Nva refers to Norvaline, Or refers to Ornithine, PheCarboxy refers to para carboxy Phenylalanine, PheCl refers to p chloro Phenylalanine, PheF refers to para fluoro Phenylalanine, PheMe refers to para methyl Phenylalanine, $PheNH_2$ refers to para amino Phenylalanine, $PheNO_2$ refers to p nitro Phenylalanine, Phg refers to Phenylglycine, Thi refers to Thienylalanine.

Pharmacology

The compounds of the present invention can be administered to a subject in a number of ways, which are well known in the art. Hereinafter, the term "subject" refer the human or lower animal to whom compounds of the present invention are administer

The novel pharmaceutical compositions of the present invention contain in addition to the active ingredient conventional pharmaceutically acceptable carriers, diluents and the like. Solid compositions for oral administration such as tablets, pills, capsules or the like may be prepared by mixing the active ingredient with conventional, pharmaceutically acceptable ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate and gums with pharmaceutically acceptable diluents. The tablets or pills can be coated or otherwise compounded with pharmaceutically acceptable materials known in the art to provide a dosage form affording prolonged action or sustained release. Other solid compositions can be prepared as suppositories, for rectal administration. Liquid forms may be prepared for oral administration or for injection, the term including subcutaneous, transdermal, intravenous, intrathecal, and other parenteral routes of administration. The liquid compositions include aqueous solutions, with or without organic cosolvents, aqueous or oil suspensions, flavored emulsions with edible oils, as well as elixirs and similar pharmaceutical vehicles. In addition, the compositions of the present invention may be formed as aerosols, for intranasal and like administration. More preferred formulations include sustained release or depot formulations which may provide a steady state pharmacokinetic profile.

However, it is evident to the man skilled in the art that dosages would be determined by the attending physician, according to the disease to be treated, method of administration, patient's age, weight, contraindications and the like.

All the compounds defined above are effective as inhibitors of protein kinase and can be used as active ingredients of pharmaceutical compositions for treatment of one, or simultaneously several, symptoms of the disorders defined above.

The compounds of the present invention are administered for the above defined purposes in conventional pharmaceutical forms, with the required solvents, diluents, excipients, etc. to produce a physiologically acceptable formulation. They can be administered by any of the conventional routes of administration.

It will be appreciated that the most appropriate administration of the pharmaceutical compositions of the present invention will depend on the type of disorder or disease being treated.

Chemistry:

Known inhibitors of protein kinases were used to perform a preliminary screen for PKB inhibition. Among these known protein kinase inhibitors, the known PKA inhibitor

compound H-89 was unexpectedly found to inhibit PKB activity with an IC_{50} of 2.4 μM, while inhibiting PKA activity with IC_{50} of 48 nM and PKC activity with IC_{50} of 31 μM.

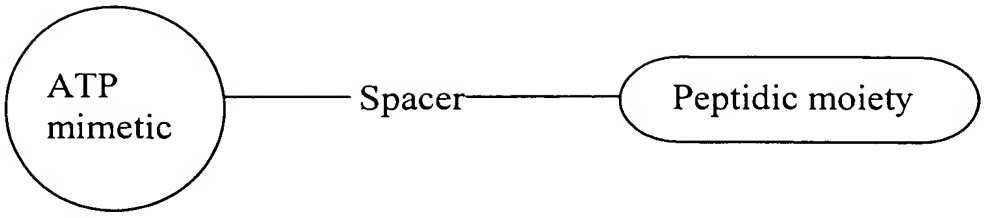
The disclosed compounds of the present invention were identified following a structure-activity relationship study involving rational and combinatorial modification of N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide also known as H-89 (Chijiwa et al. J. Biol. Chem. 265, 5267, 1990).

Some of the preferred compounds of the present invention may conveniently be prepared using solution phase synthesis methods. Other methods known in the art to prepare isoquinoline compounds like those of the present invention, can be used and are comprised in the scope of the present invention. Preferred peptides according to the present invention may be synthesized using any method known in the art. These methods include solid phase as well as solution phase synthesis methods.

By way of exemplification of the principles of the present invention, a search for inhibitory PKB compounds focused on SAR studies of the H-89 molecule, as exemplified hereinbelow. This was followed by SAR study of additional known protein kinase inhibitors that were unexpectedly identified as PKB inhibitors.

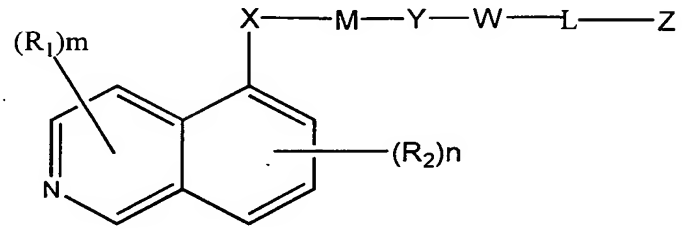
Protein kinases have more than one active site, they possess a catalytic site for ATP and a substrate binding site. Additional preferred compounds according to the present invention can bind both sites at the same time and may have a synergistic effect that will give it unique potency and selectivity properties. These preferred compounds are chimeric molecules which are designed to include an ATP-mimetic molecule, connected via various spacers to a substrate-mimetic portion.

Preferred embodiments according to the present invention comprise a chimeric compound comprising both an ATP mimetic moiety and a peptidic substrate, as described in the following scheme:



The ATP mimetic core includes but is not limited to dansyls, isoquinolines, quinolines and naphthalenes. The spacer is of varied lengths and conformations of any suitable chemistry including but not limited to amine, amide, thioether, oxyether, sulfonamide bond and the like. Non limiting examples for such spacers include sulfone amide derivatives, amino thiol derivatives and amino alcohol derivatives. The peptidic moiety comprises peptides and peptidomimetics. Such inhibitory peptides may be designed based on any peptide which may serve as PKB substrate.

Additional more preferred embodiment of the present invention comprises a compound of Formula I



Formula I

wherein:

R_1 and R_2 are independently selected from the group consisting of hydrogen, a lower alkyl group, a lower alkoxy group, substituted or unsubstituted phenyl group, a lower alkyl substituted with at least one substituent selected from the group consisting of a phenyl group, a halogen, hydroxyl, thiol, nitro, cyano, or amino group;

m and n are each independently 0-3;

X is selected from the group consisting of SO_2-NH, S and O;

M represents substituted or unsubstituted alkylene of 1-4 carbon atoms;

Y is selected from the group consisting of amide, amine, urea, carbamate, hydrazine or sulfonamide;

W is absent or is selected form the group consisting of substituted or unsubstituted alkylene, aliphatic, aromatic or heterocyclic moiety, of 1-18 carbon atoms;
L is absent or is selected from the group consisting of amide, amine, urea, carbamate, hydrazine or sulfonamide; and
Z is a peptide or peptidomimetic moiety of 4-12 residues in length capable of binding to the substrate site of PKB.

Preferred peptide substrate and peptide substrate mimetics according to the present invention, forming part of the chimeric compounds are described in the following scheme:

AA_1—— AA_2—— AA_3—— AA_4—— AA_5—— AA_6——AA_7

According to this scheme AA is selected from the group consisting of an amino acid, an amino acid analog, or an aliphatic, aromatic or heterocyclic moiety, incorporated into the sequence to create a peptidomimetic moiety with improved pharmacological properties.

AA_1 and AA_3 are independently selected from the group consisting of: arginine or arginine analog; lysine or lysine analog; ornithine or ornithine analog; or an aliphatic, aromatic, or heterocyclic moiety bearing a group positively charged at physiological pH, such as an amine, guanidine or amidine, homoarginine, argininol.

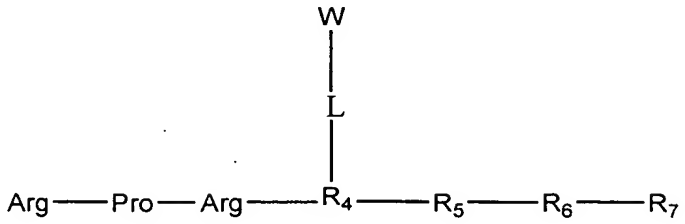
AA_2 is selected from the group consisting of proline, proline analog or an aliphatic, aromatic or heterocyclic moiety, hydroxyproline, nipecotic acid, alanine, aminobutyric acid.

AA_4, AA_5, AA_6 are independently selected from the group consisting of: Thr or Thr analog; Ser or Ser analog; Ala or Ala analog; Glu or Glu analog; an aliphatic, aromatic or heterocyclic residue bearing alkyl, benzyl, hydroxy, phenoxy alkoxy, sulfone, sulfoxide, phosphonate, phosphonate ester, amide or carbamoyl functionality, amino butyric acid, citrulline, serinol, phosphotyrosine and phosphotyrosine dimethyl ester.

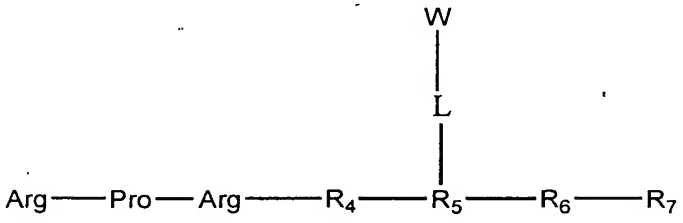
AA_7 is selected from the group consisting of: Phe or Phe analog; Trp, Tyr, Leu, Ile, and their analogs; aromatic moiety esters or aromatic substitutions of an amino acid; an aromatic, heterocyclic or branched aliphatic moiety; homophenylalanine, homoleucine, glutamic benzyl ester, naphtylalanine.

Due to the peptidomimetic nature of the preferred embodiments according to the invention, the bonds between AAs are not only peptide bonds but may be selected from the group consisting of: an amide, urea, carbamate, hydrazine or sulfoneamide bond.

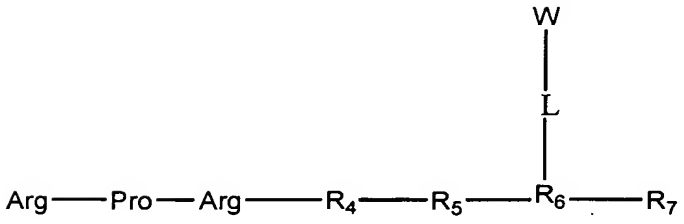
Additional preferred embodiments of the present invention comprise a compound of Formulae IIa - IId:



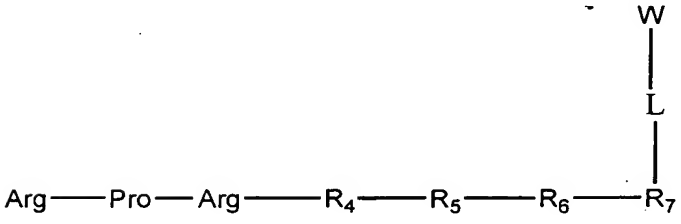
Formula IIa



Formula IIb



Formula IIc



Formula IId

wherein:

R_4 is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R_5 and R_6 are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

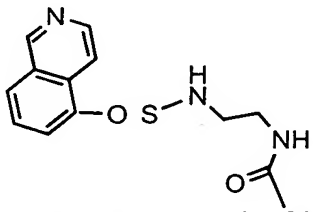
R_7 is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R_7 represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid.

One currently most preferred embodiment of the present invention is the chimeric compound denoted hereinbelow as PTR 6016 described in Formula III:



Arg-Pro-Arg-Thr-Glu-Ser-Phe

Formula III

Figure 1 describes the synergistic effect of conjugation of the ATP mimic part which does not inhibit PKB, with the peptide substrate part having activity of 4 μM, to yield the chimeric compound PTR 6016 with activity of 0.9 μM activity.

Additional chimeric compounds comprising active peptides with diverse linkers connecting the peptidic moiety region to the spacer (according to scheme 1). The favorable linker enables simultaneous fit of both the peptide and ATP mimic into their substrate and ATP binding sites, and improvement of activity and specificity.

Additional preferred peptides according to the present invention may be uses as the substrate domain of the chimeras and a basis for design of peptidomimetics. For example, linear 7-mer peptides with IC_{50} of 4-5 μM for PKB are disclosed in comparison to the reference 7-mer substrate that has K_m of 15 μM. These peptides are specific to PKB and do not inhibit PKA activity at 60 μM. Additional peptides disclosed demonstrate activity of 0.5-10 μM for inhibition of PKB.

Peptidomimetic compounds having improved stability and cell permeability properties are another embodiment of the present invention. Non limiting examples of generation of such compounds include N alkylation of selected peptide residues, carbamate, urea and hydrazine bond replacement, and incorporation of hetrocyclic non-peptide moieties such as piperidine, piperazine, pyrrolidine etc. through peptide or non-peptide bond.

Biological screening assays for Inhibition of Protein Kinase activity:

Inhibition of enzyme activity in cell free system: PKA activity assay:

PKA activity is assayed on a 7-mer peptide, LRRASLG, known as kemptide. The assay is carried out in 96-well plates, in a final volume of 50 μl per well. The reaction mixture includes various concentrations of the inhibitor, 50 mM MOPS, 10 mM MgAc, 0.2 mg/ml BSA, 10 μM ATP, 20 μM Kemptide and 1μCi $\gamma^{32}P$ ATP. Reaction is started with addition of 15 μl of the catalytic subunit of PKA diluted in 0.1 mg/ml BSA, 0.4 U/well. Two blank wells without enzyme are included in every assay. The plates are agitated continuously at 30°C for 10'. Reaction is stopped by addition of 12 μl 200 mM EDTA. 20 μl aliquots of the assay mixture are spotted onto 2 cm^2 phosphocellulose strips (e.g. Whatman P81) and immersed in 75 mM phosphoric acid (10 ml per sample). The phosphocellulose strips are washed 6 times. Washes are done in continuous swirling for 5 minutes. last wash is in acetone. After air drying the strips, radiation is measured by scintillation spectrometry. Screening of libraries is done in duplicates with a single concentration of the inhibitor (5 μM). purified compounds are checked in various concentrations and their IC_{50} value is determined.

Inhibition of enzyme activity in cell free system: PKB activity assay:

PKB activity is assayed as described in Alessi et al. (FEBS Letters 399, 333, 1996) with the following modifications: instead of HA-PKB coupled to beads, soluble His-HA-PKB is used following precipitation on a Nickel column. The enzyme activity measurement is performed as described in the assay for PKA.

Assays for inhibition of PKB activity in intact cells:

Several cancer cell lines were used to determine the activity of PKB inhibitors in intact cells. For example OVCAR3 is a cell line of ovarian carcinoma with an amplification of the PKB gene, U87MG is a glioma cell line with a deletion of PTEN gene – causing high activity of PKB, and PANC1 is a pancreatic carcinoma cell line with an amplification of PKB gene.

a. Annexin-V assay for apoptosis:

OVCAR3 cells were seeded in 10 cm plates ($2x10^6$ cells/plate) and treated with different concentrations of the inhibitor. 40 hours after treatment cells were trypsinysed, washed twice with PBS and suspended in annexin-V buffer. annexin-V (Roche) is diluted 1:250 in a buffer containing 10 mM HEPES pH 7.4, 140 mM NaCl, 5 mM $CaCl_2$ and 0.2 nM propidium iodid (PI). Apoptosis measurement was performed by FACS analysis.

b. Growth inhibition:

OVCAR3, U87MG and PANC1 cells were seeded in 96-well plates. In each plate cells were treated with different concentrations (0, 5, 10, 25, 50, 75, 100 μM) of the inhibitor, in triplicates. Every day one plate was fixed by 0.5% gluterdialdehyde, and the inhibitor was replaced in the rest of the plates. After fixation the cells were stained with methylene-blue 1% for one hour. Plates were washed with distilled water and dried. Extraction of color was performed by adding 0.1 M HCl for one hour at 37°C. Quantitation of color intensity was performed by measurement of the optical density at 620 nm by ELISA reader.

c. Inhibition of phosphorylation:

Cells were seeded in 6-well plates, and treated with different concentrations of the inhibitor. Treatment was taken either under serum containing media or under starvation for different time periods. After treatment cells are stimulated for 10' with IGF-1 (HEK-293

and PANC1 cells) or EGF (OVCAR3 and U89MG cells). Cell lysates are prepared using boiled sample buffer. Western blot analysis with α phospho-GSK3 showed decrease in GSK3 phosphorylation. The effect was also tested on GSK3 phosphorylation by expression of kinase-dead-PKB in 293 cells.

Transfer ELISA assay for measuring PKB activity and inhibition.

The inhibitor tested is dissolved in water to the desired concentration. Five µl of the inhibitor solution is added to the wells of a V shaped polyproplylene microplate. Five µl of substrate peptide (Biotin-Lys-Gly-Arg-Pro-Arg-Thr-Ser-Ser-Phe-Ala-Glu-Gly) solution in water at a concentration of 300 µM is then added to the wells (final assay concentration is 100 µM). Then PKB enzyme dissolved in 3x reaction mixture (50 mM Tris HCl pH 7.5, 0.1% beta mercaptoethanol, 1 µM PKI (Calbiochem), 10 mM Mg acetate, ATP 5 µM), is added in pre-calibrated amount to the wells. The amount of enzyme is calibrated so that less than 10% of the substrate is phosphorylated by the end of the reaction as evaluated by mass spectral analysis. The plate is covered with an adhesive tape, placed over a 1 mm ID vortex at 30°C and incubated for 30 min to 1 hour as needed. At the end of the incubation period 5 µl of 0.5 M disdium EDTA are added to the wells followed by 180 µl of PBS .

For ELISA, a microplate (Costar A/2) is coated with 20 µl of 10 µg/ml of avidin in PBS (over night at 4°C or 30 minutes at 37°C, on a 1 mm ID vortex). The plate is than washed several times with dionized water and flicked dry on a towel paper. The wells are filled with 20 µl of PBT (PBS + 1% BSA + 0.05% tween 20). Five µl from the enzyme reaction plate are transferred to the ELISA plate. The ELISA plate in placed on the 1 mm ID vortex and incubated for 10 min at RT. The plate is than washed with water as before. To each well 20 µl of anti phosphopeptide antibody (Cell Signaling Technology) diluted 1:1000 in PBT are added. The plate is placed again on the vortex, incubated for 30 minutes and washed with water as before. To each well 20 µl of goat anti- rabbit Ig conjugate with horse raddish peroxidase (HRP) is added. The plate is placed on the vortex, incubated for 20 min and washed with water as before. To each well is added 20 µl of HRP substrate (Sigmafast OPD). After sufficient color development (up to maximum of about 30 minutes development time) the reaction is terminated by the addition of 20 µl per well of 4 M HCl in water. The plate is than read using an ELISA reader at 490 nm. The signal obtained from wells containing potential inhibitors is compared to signal obtained from wells

containing only the enzyme without inhibitor (maximum signal) and wells not containing enzyme (minimum signal).

The fraction of phosphorylated peptide can be also analyzed by mass spectra following desalting on a ziptip (C18, Millipore i). Mass of double charged substrate peptide is 759.3 Dalton, and of the double charged phosphorylated peptide is 799.3 Dalton.

Synthetic methods:

General methods for synthesis of peptides and carbamate bond formation.

The following procedure describes the synthesis of peptides in 96 wells plate (MPS plate) at a scale of 6 μmol peptide per well, on Rink amide resin, using bis-(trichloromethyl)carbonate (BTC) for carbamate formation and HBTU/HOBT for normal coupling.

One gram of rink amide 0.6 mmol/g was swelled overnight in NMP with gentle shaking. The resin was distributed ino 96 wells plate (~10 mg per well).

Fmoc deprotection performed by adding 500 μl of 25% piperidine solution in NMP to each well and mixing at 650 rpm for 15 min, the pipridine solution is removed by a pressure of nitrogen and another portion of piperidin solution is added and shacked for 15 min. Wash of resin after Fmoc deprotection and after couplings, performed by placing 600 μl NMP into each well, mixing for 2 min. and removing the NMP by nitrogen pressure. The washing procedure is repeated four times.

Regular coupling is performed by adding a solution of Fmoc protected amino acids (150 μl, 0.2M) in HOBT/NMP to the resin, followed by addition of HBTU solution in DMF (150 μl, 0.2M) and DIEA in NMP (150 μl, 0.4M). The reaction vessel block is mixed at 650 rpm for 1h and then removed by a pressure of nitrogen. This procedure is repeated once.

Carbamate formation using BTC is performed by addition of a solution of Fmoc protected amino alcohols in dioxane (150 μl, 0.2 M), to a preactivation deep well plate, followed by addition of 150 μl BTC (0.07 M) in 1,3-dichloropropane, 150 μl collidine (0.6 M) in 1,3-dichloropropane and 200 μl CH_2Br_2. The isocyanate solution is then transferred into the reaction vessel block and mixed for 40 min at 60^0C. After 40min the reaction mixture is removed by nitrogen pressure followed by CH_2Cl_2 wash (3x 400 μl). This procedure is repeated for additional two times.

Cleavage and global deprotection are performed by transferring the resin from the reaction vessel block into a deep well microtitier plate (cleavage plate). To this plate 350 µl solution of 92.5% TFA, 2.5% H_2O, 2.5% TIS, 2.5% EDT is added. The plate is mixed at 1000 rpm for 1h and then the TFA solution is evaporated to dryness.

Purification by Sep-Pak performed by dissolving the residue of the resin with the peptide in 900 µl solution A (0.1% TFA in water) and applying on C-18 Sep-Pak column. The peptides are eluted from the C-18 column by addition of 900 µl solution A + CH_3CN 1:1 to a deep well plate. The plate is frozen in liquid nitrogen at least 15 min and the peptides are lyophilized.

The skilled artisan will appreciate that the following examples are merely illustrative and serve as non limitative exemplification of the principles of the present invention and that many variations and modifications are possible within the scope of the currently claimed invention as defined by the claims which follow.

EXAMPLES

Example 1. Screening PKA inhibitors for PKB inhibition

Since there are no known inhibitors of PKB, the structural similarity between PKB and other protein kinases was used to screen commercially available inhibitors of other protein kinases, e.g., PKA and PKC, for PKB inhibition. The preliminary screen was conducted in order to define some structural motifs in active compounds that would assist in the initial design of a combinatorial library of candidate compounds.

It should be noted, however, that though this approach is very useful for rapid identification of lead molecules, the molecules that are identified would possess inhibition activity against other kinases as well. Thus, this approach dictates research directed not only at optimization of the inhibitory activity, but also, and perhaps most importantly, specificity-oriented research. Namely, substantial efforts are actually directed at modifying the selectivity profile, in order to obtain a profile of selectivity or specificity towards PKB.

The screen yielded two compounds that inhibited PKB in the 2-3 µM range. H-89, a known PKA inhibitor, was chosen to be the basic scaffold for the design of the first library, based on its structure and on synthetic and specificity considerations.

H-89 was further optimized using rational design and parallel synthesis methods as described in Israeli application No. 136458. It was concluded that the 5-isoquinoline-sulfonamide-ethylenediamine core is essential for activity and replacement with any other core, either as a sulfonamide or a carboxamide derivative, eliminated activity. The substrate mimetic region C was also studied and it what concluded that this region could contain a hydrophobic or heterocyclic moiety or a peptide capable of binding PKB. Outline of these findings is given in the following example.

Example 2. Modification of H-89 for identification of PKB inhibitors

The structure of H-89 makes it an ideal candidate for SAR study using combinatorial chemistry, since it allows diversity in many regions of the molecule. The isoquinoline moiety could be replaced with various bicyclic and aromatic residues, the ethylendiamine bridge can vary in length, hydrogen bonding properties and substitution, and the cinnamoyl moiety can be modified to a large variety of structures for the evaluation of the optimal properties of this region. In addition, the sulfonamide group can be replaced with carbonyl and other similar moieties. There are various regions of diversity in the structure of H-89 which were used to construct combinatorial libraries. The libraries were designed to explore each region's contribution to the inhibition potency and to the specificity against other kinases.

Region A. A diversity of bicyclic cores, and aromatic heterocyclic cores.

Region B. A diversity of spacers modified in length, electrostatic properties and substitutions.

Region C. A diversity of "tails", either aliphatic or aromatic, differing in length, electrostatic and steric properties.

Region D. Replacement of the sulfonamide with a carboxy amide, urea, amine, a simple methylene, etc.

Analysis of the SAR results clearly shows that at region A, none of the cores that were tested worked, except of the 5-isoquinoline. Replacement with any other core, either as a sulfonamide or a carboxamide derivative, eliminated activity. On region B, elongation of the bridge from two to three carbons diminished activity, as did substitution on the chain. Using cyclic secondary amine derivatives resulted in elimination of the activity in the case of homopiperazine, implying that the amino protons are important to the interaction,

possibly through hydrogen bonding. Surprisingly, the piperazine derivatives showed activity, but significantly less pronounced than the corresponding ethylenediamine analogs.

The most significant SAR was observed in the diversity of moieties used for region C. The results show that activities of compounds derived from 5-isoquinoline-sulfonamide-ethylenediamine, with various moieties at region C, vary from almost no activity to very significant activities. Notably, significant variation was observed also in the activity of PKA. In several cases, the inhibition of PKA was significantly decreased. Several of the active compounds from the library were selected for re-synthesis, purification and full characterization, and IC_{50} values for inhibition of PKA and PKB were determined.

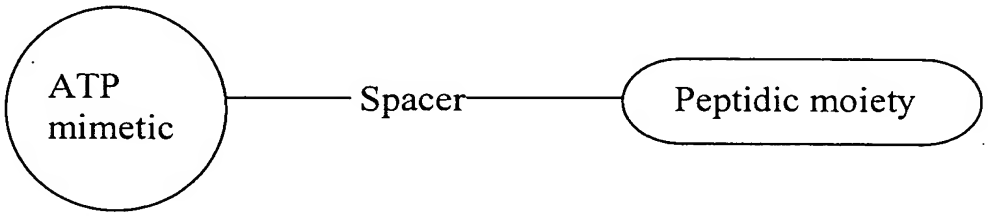
As discussed before, identifying non-mutual interactions was important for the design of specific inhibitors. The SAR results indicate that an additional bulky substitution at the carbon bearing the aromatic residue in region C, is unfavorable for PKA inhibition, but has a negligible effect on PKB inhibition.

To verify this conclusion, we synthesized a series of compounds bearing such a bulky substitution. The results demonstrate that molecules with additional hydrophobic substitution next to the aromatic residue in region C showed significant decrease in PKA inhibition, and a very small effect on PKB inhibition. These results confirm that this position is indeed a PKA "irritant", shifting its IC_{50} value for 50 nM to several μM, while its effect on PKB is minor. The identification of this feature is crucial to the further optimization of the disclosed compounds . Since the two enzymes are so similar, a major potential problem is that any modification we make that will improve affinity for PKB, will probably have the same effect on PKA. However, by having the ability to introduce a bulky substitution at the identified position we can selectively decrease the activity of PKA with only negligible effects on the activity of PKB.

Example 3. Chimeric compounds having ATP and substrate mimetic sites.

Chimeric molecules are designed to combine an ATP- mimetic together with a substrate- mimetic regions connected via a bridge. These chimeric molecules can bind to both the catalytic site and to the substrate site of protein kinases at the same time and may have a synergistic effect that affords unique potency and selectivity properties. These compounds are designed to include an ATP-mimetic molecule, connected via various spacers to a substrate mimetic portion.

These compounds are identified following synthesis and screening cycles of combinatorial libraries in which each library examines modifications at a different region based on the following scheme:



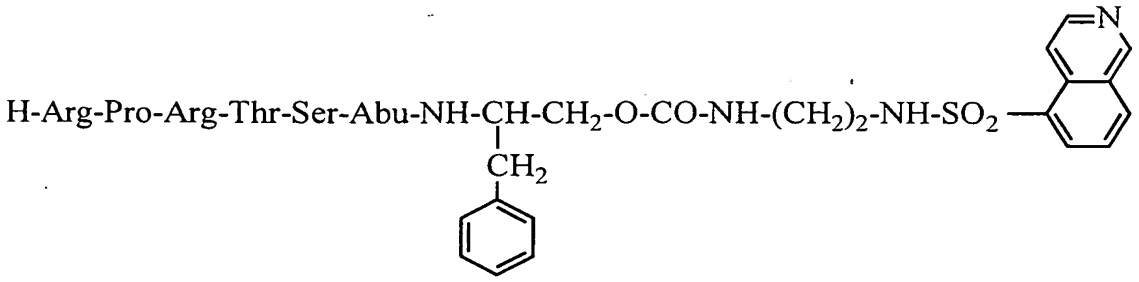
The ATP mimetic core includes but is not limited to dansyls, isoquinolines, quinolines and naphthalenes. The spacer is of varied lengths and conformations of any suitable chemistry including but not limited to amine, amide, thioether, oxyether, sulfonamide bond and the like. Non limiting examples for such spacers include sulfone amide derivatives, amino thiol derivatives and amino alcohol derivatives. The peptidic moiety comprises peptides and peptidomimetics. Such inhibitory peptides may be designed based on any peptide which may serve as PKB substrate.

Example 4. Detailed synthesis of chimeric compounds

PTR 6013

Four hundred mg of 4-(4-formyl-3-methoxyphenoxy)butyryl (NovaGel HL) were swelled for 1.5 h in dichloroethane/trimethylorthoformate (1:1) in a reactor equipped with a sintered glass bottom, attached to a shaker. 352 mg (9 equivalents) of N-(8-sulfonamide-5-isoquinoline)ethylenediamine in 12 ml DMF was added to the resin followed by addition of $NaBH(OAc)_3$ and continues shaking over night. The resin was washed with DMF followed by DCM. Formation of the carbamate bond was performed by addition of Fmoc-Phenyalaninol (291 mg, 5 equivalents) which was preactivated with BTC (77 mg, 1.66 equivalents) and 2,4,6- collidine (290 µl, 14 equivalents) in THF twice at 50^0C. Fmoc was removed from the resin using 25% Piperidine in NMP (3 ml) twice for 15 min followed by careful wash, seven times with NMP (5 ml), for 2 min each. Assembly of Abu, Ser, Thr, Arg, Pro Arg was accomplished by coupling cycles using Fmoc-Abu-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, and Fmoc-Pro-OH respectively. In each coupling cycle the amino acid (3 equivalents) was dissolved in NMP and was activated with PyBroP (3 equivalents) and DIEA (6 equivalents). Following coupling, the peptide-resin was washed, than Fmoc was removed followed by extensive

wash with NMP, as described above for the first coupling. At the end of the assembly the peptide was cleaved from the resin using 65% TFA, 20% DCM, 5% thioanisole, 3% EDT, 2% TIS and 5% water in a total volume of 7 ml cocktail mixture for 15 min at 0^0C under Argon and then 2h at room temperature. The solution was filtered through extract filter into polypropylene tube, the resin was washed with 3 ml of 60% TFA in DCM, the combined solution was evaporated by N_2 stream to give oily residue which on treatment with cold Et_2O solidify. Centrifugation and decantation of the Et_2O layer and treatment with additional portion of cold Et_2O followed by centrifugation, decantation and drying of the white solid under vacuum over night, gave crude material denoted PTR 6013 having the following structure:

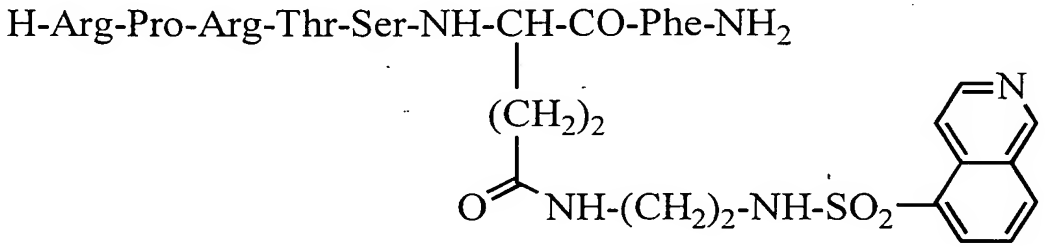


PTR 6013

PTR 6014

Five hundred mg of Rink amide MBHA resin (0.55 mMol/g) were swelled for 2 h in NMP in a reactor equipped with a sintered glass bottom, attached to a shaker. Fmoc was removed from the resin using 25% Piperidine in NMP (4 ml) twice for 15 min followed by careful wash, seven times with NMP (5 ml), for 2 min each. Assembly of Phe, Glu, Ser, Thr, Arg, Pro, Arg was accomplished by coupling cycles using Fmoc-Phe-OH, Fmoc-Glu(OAllyl)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, and Fmoc-Pro-OH respectively. In each coupling cycle, the amino acid (3 equivalents) was dissolved in NMP and was activated with PyBroP (3 equivalents) and DIEA (6 equivalents). At the end of assembly allyl deprotection took place using $Pd(PPh_3)_4$ in solution of CH_2Cl_2 containing 5% AcOH and 2.5% NMM. The free acid was activated by 3 equivalents PyBoP and 3.1 equivalents DIEA in NMP for 20 min followed by NMP wash. After preactivation a solution of small molecule (3 equivalents) and DIEA (4.5 equivalents) in NMP was added to the resin and shaken for 1h at room temperature. Following coupling, the peptide-resin was washed with NMP, than Fmoc was removed followed by extensive wash with NMP, as described above for the first coupling. At the end of the synthesis the peptide was cleaved

from the resin using 85% TFA, 5% thioanisole, 3% EDT, 2% TIS and 5% water in a total volume of 5 ml cocktail mixture for 15 min at 0^0C under Argon and then 2h at room temperature. The solution was filtered through extract filter into polypropylene tube, the resin was washed with 2 ml of TFA. The combined solution was evaporated by N_2 stream to give oily residue, which on treatment with cold Et_2O solidify. Centrifugation and decantation of the Et_2O layer and treatment with additional portion of cold Et_2O followed by centrifugation, decantation and drying the white solid under vacuum over night gave crude material denoted PTR 6014 having the following structure:

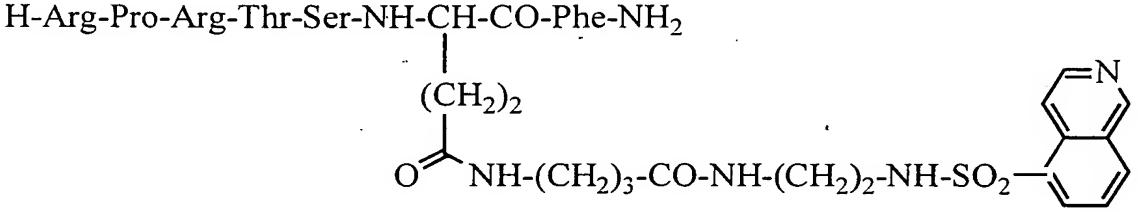


PTR 6014

PTR 6020

Five hundred mg of Rink amide MBHA resin (0.55 mMol/g) were swelled for 2 h in NMP in a reactor equipped with a sintered glass bottom, attached to a shaker. Fmoc was removed from the resin using 25% piperidine in NMP (4 ml) twice for 15 min followed by careful wash, seven times with NMP (5 ml), for 2 min each. Assembly of Phe, Glu, Ser, Thr, Arg, Pro, Arg was accomplished by coupling cycles using Fmoc-Phe-OH, Fmoc-Glu(OAllyl)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, and Fmoc-Pro-OH respectively. In each coupling cycle the amino acid (3 equivalents) was dissolved in NMP and was activated with PyBroP (3 equivalents) and DIEA (6 equivalents). At the end of assembly allyl deprotection took place using $Pd(PPh_3)_4$ in solution of CH_2Cl_2 containing 5% AcOH and 2.5% NMM. The free acid was activated by 3 equivalents PyBOP and 3.1 equivalents DIEA in NMP for 20 min followed by NMP wash. After preactivation a solution of Allyl γ-aminobutyrate (5 equivalents) and DIEA (6 equivalents) in NMP was added and shaken for 1h at room temperature. The allyl deprotection and preactivation was performed by the same procedure as described above. A solution of the small molecule (3 equivalents) and DIEA (4.5 equivalents) in NMP was added to the preactivated peptide-resin and shaken for 1h at room temperature. Following coupling, the peptide-resin was washed with NMP, than Fmoc was removed followed by extensive wash with NMP, as

described above for the first coupling. At the end of the synthesis the peptide was cleaved from the resin using 85% TFA, 5% thioanisole, 3% EDT, 2% TIS and 5% water in a total of 5 ml cocktail mixture for 15 min at 0^0C under Argon and then 2h at room temperature. The solution was filtered through extract filter into polypropylene tube, the resin was washed with 2 ml of TFA, the combined solution was evaporated by N_2 stream to give oily residue which on treatment with cold Et_2O solidify. Centrifugation and decantation of the Et_2O layer and treatment with additional portion of cold Et_2O followed by centrifugation, decantation and drying the white solid under vacuum over night gave crude material denoted PTR 6020 having the following structure:



PTR 6020

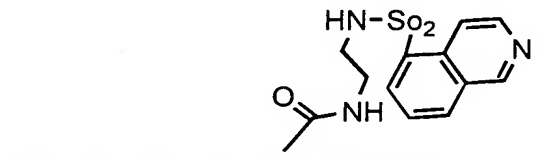
Example 5. Biological activity of chimeric compounds

Four chimeric compounds were screened for PKB inhibition activity. Table 3 describes their structure and inhibition activities. Similar to the compound denoted B-11-1 these compounds are not specific for PKA.

Table 3.

ID	activity PKB	Activity PKA
PTR 6013	3μM	1μM
PTR 6014	25μM	NA
PTR 6016	0.9μM	0.5μM
PTR 6020	> 20μM	NA

Structures of PTR 6013, 6014 and 6020 are described in example 7. The structure of PTR 6016 is:



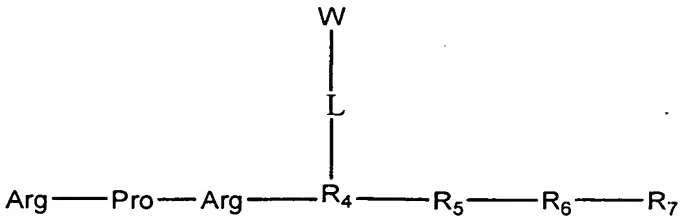
PTR 6016

Figure 1 describes the synergistic effect of conjugation of the ATP mimic part which does not inhibit PKB, with the peptide substrate part having activity of 4 μM, to yield the chimeric compound PTR 6016 with activity of 0.9 μM activity.

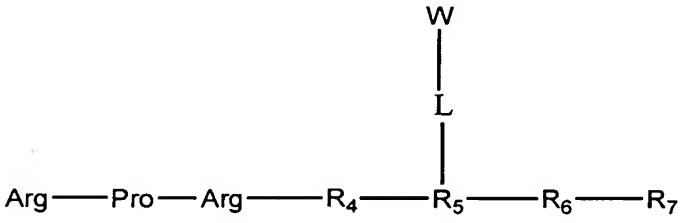
Example 6. Additional chimeric compounds

A multiple-parallel-synthesis plate including 96 chimeric compounds of active peptides from plates 6002, 6003 (region A in scheme I) with diverse linkers connecting the peptide region C to the di-amino bridge B, was synthesized. These peptides were designed for elucidating the appropriate linker which enables simultaneous fit of both the peptide and ATP mimic into their substrate and ATP binding sites, for improving activity and specificity.

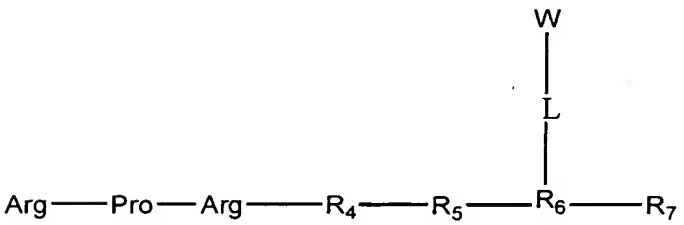
The compounds synthesized are described in formulae IIa-IId:



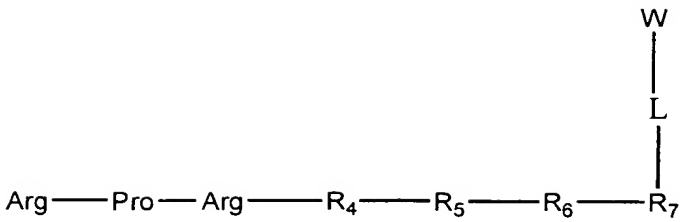
Formula IIa



Formula IIb



Formula IIc



Formula IId

wherein:

R_4 is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R_5 and R_6 are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

R_7 is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R_7 represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid.

Example 7. Peptides

Additional peptides are designed for use in the substrate domain of the chimeras and for design of peptidomimetics. Two plates of linear 7-mer peptides were synthesized and purified. Four peptides from the first plate (6002) were found to be active with IC_{50} of 4-5 μM for PKB (the reference 7-mer substrate ahs IC_{50} of 15 μM). All these peptides do not inhibit PKA activity at 60 μM. From the second plate (6003) ~ 24 active peptides were identified, peptides exhibiting >60% inhibition were tested again, demonstrating activity of 0.5-10 μM for PKB. Specificity to PKA not tested yet. Additional 1152 peptides from macrobeads library were screened, 150 had over 50% inhibition at 10 μM. Three multiple-parallel-synthesis plates were then planed and synthesized. Selected results are presented in the following table.

Table 4.

ID	structure	Activity PKB	Activity PKA
TY 60002-50:	Arg-Pro-Arg-Thr-Ser-Ala-Hol	5μM	>40μM
TY 60002-61:	Arg-Pro-Arg-Val-Ser-Abu-Phe	5μM	>40μM
TY 60002-73:	Arg-Pro-Arg-Thr-Ser-Abu-Hol	5μM	>40μM
TY 60002-96:	Arg-Pro-Arg-Thr-Ser-Dap-Hol	5μM	>40μM
TY 60002-18	Arg-Pro-Arg-Thr-Ser-Asp-Phe	Not active	
AR 60003-50:	Arg-Pro-Arg-Met-Ser-Ser-Phe	2.5μM	
AR 60003-52:	Arg-Pro-Arg-Orn-Ser-Ser-Phe	2.5μM	
AR 60003-53:	Arg-Pro-Arg-Arg-Ser-Ser-Phe	3μM	
AR 60003-62:	Arg-Pro-Arg-Nle-Ser-Ser-Nle	<1μM (70% inhibition at 1μM)	
AR 60003-64	Arg-Pro-Arg-Arg-ser-Ser-Arg	Not active	
AR 60003-96	Arg-Pro-Arg-Orn-Ala-Thr-Orn	Not active	

The PKB inhibition activity of peptide AR-60003-52 as determined in ELISA is illustrated in figure 2.

Example 8. Peptidomimetic compounds based on the active peptides:

The following peptidomimetic compounds which contains carbamate and/or urea bonds replacing peptidic bonds, were synthesized.

PTR 6046: H-Arg-NH-$(CH_2)_2$-NH-CO-Arg-Thr-Ser-Dap-Hol-NH_2

PTR 6048: H-Arg-NH-CH_2-C_6H_4-CH_2-NH-CO-Arg-Thr-Ser-Dap-Hol- NH_2

PTR 6050: H_2N-$(CH_2)_4$-NH-CO-Pro-Arg-Thr-Ser-Dap-Hol- NH_2

PTR 6052: H_2N-$(CH_2)_4$-NH-CO-3-(HNCH_2)-C_6H_4-CH_2NH-CO-Arg-Thr-Ser-Dap-Hol-NH_2

PTR 6054: H-ArgOl-NH-CH_2-C_6H_4-CH_2-NH-CO-Arg-Thr-Ser-Dap-Hol-NH_2 (this compound contains both carbamate and urea bonds).

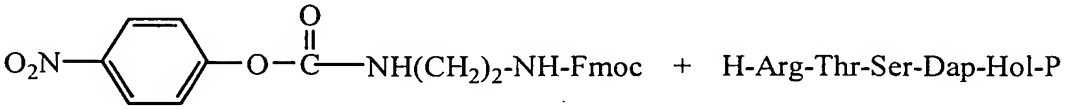
PTR 6056: H-Arg-1,4-Homopiperazine-CO-Arg-Thr-Ser-Dap-Hol-NH_2

Example 9. Detailed synthesis of PTR 6046, an heptamer containing an urea bond

One hundred mg (0.055 μmol) of rink amide resin were swelled 1.5h in NMP in a reactor with a sintered glass bottom, attached to a shaker. Fmoc was removed from the resin using 25% piperidine in NMP (3 ml) twice for 15 min followed by careful wash, seven times with NMP (2 ml). Assembly of Arg-Thr-Ser-Dap-Hol was accomplished by coupling cycles using Fmoc-Hol-OH, Fmoc-Dap-OH, Fmoc-Ser(tBu)-OH,

Fmoc-Thr(tBu)-OH, Fmoc-Arg(Pmc)-OH. In each coupling cycle the amino acid (3 equivalents) was dissolved in NMP and was activated with PyBroP (3 equivalents) and DIEA (6 equivalents). Following coupling, the peptide-resin was washed, than Fmoc was removed followed by extensive wash with NMP.

Formation of the urea bond:



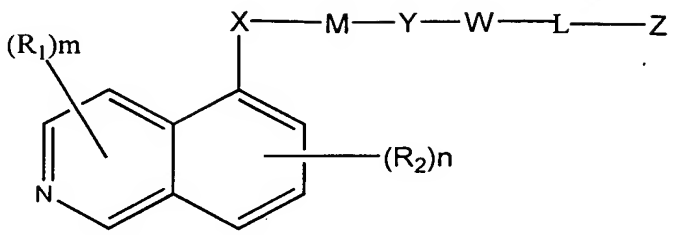
50 mg (2 equivalents) of N-Fluorenylmethoxycarbonyl-N'-netrophenoxycarbonyldiaminoethane, 25 µl (2.5 equivalents) of DIEA in 2 ml NMP was added to the resin and continues shaking 1.5h. The resin was washed with NMP (5 times 2 min each). After formation of the urea bond, a coupling of Fmoc-Arg(Pmc)-OH was performed as describe above followed by Fmoc deprotection. At the end of the assembly the peptide was cleaved from the resin using 92.5% TFA, 2.5% EDT, 2.5% TIS and 2.5% water in a total volume of 5 ml cocktail mixture and continues shaking 1 h .The solution was filtered through extract filter into polypropylene tube, the resin was washed with 2 ml of TFA, the combined solution was evaporated by N_2 stream to give oily residue which on treatment with cold Et_2O solidify. Centrifugation and decantation of the Et_2O layer and treatment with additional portion of cold Et_2O followed by centrifugation and decantation and drying the white solid under vacuum over night gave crude PTR 6046 having the following structure:

H-Arg-NH-$(CH_2)_2$-NH-CO-Arg-Thr-Ser-Dap-Hol-NH_2

CLAIMS

What is claimed is:

1. A compound of Formula I:



Formula I

wherein:

R_1 and R_2 are independently selected from the group consisting of hydrogen, a lower alkyl group, a lower alkoxy group, substituted or unsubstituted phenyl group, a lower alkyl substituted with at least one substituent selected from the group consisting of a phenyl group, a halogen, hydroxyl, thiol, nitro, cyano, or amino group;

m and n are each independently 0-3;

X is selected from the group consisting of SO_2-NH, S and O;

M represents substituted or unsubstituted alkylene of 1-4 carbon atoms;

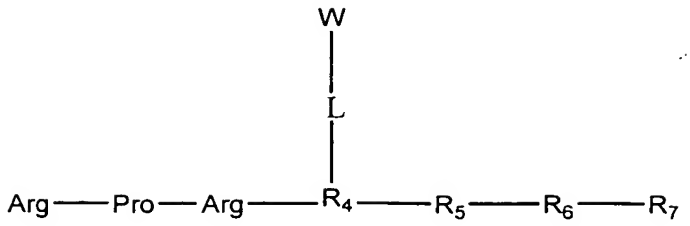
Y is selected from the group consisting of amide, amine, urea, carbamate, hydrazine or sulfonamide;

W is absent or is selected form the group consisting of substituted or unsubstituted alkylene, aliphatic, aromatic or heterocyclic moiety, of 1-18 carbon atoms;

L is absent or is selected from the group consisting of amide, amine, urea, carbamate, hydrazine or sulfonamide; and

Z is a peptide or peptidomimetic moiety of 4-12 residues in length capable of binding to the substrate site of PKB.

2. The compound of Formula IIa:



Formula IIa

wherein:

R_4 is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R_5 and R_6 are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

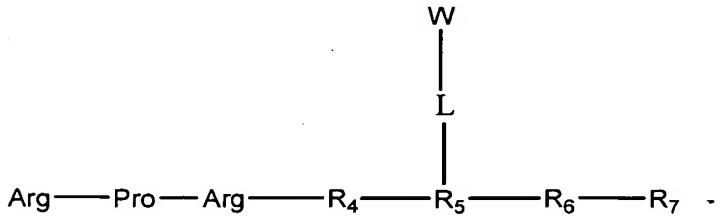
R_7 is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R_7 represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid.

3. The compound of Formula IIb:



Formula IIb

wherein:

R_4 is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R_5 and R_6 are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

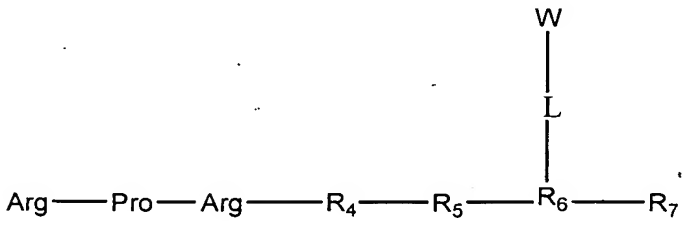
R_7 is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R_7 represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid.

4. The compound of Formula IIc:



Formula IIc

wherein:

R_4 is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R_5 and R_6 are selected from the group consisting of Serine, Glutamic acid allyl ester,. and alanine;

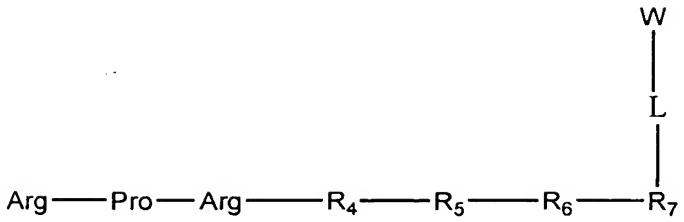
R_7 is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R_7 represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid.

5. The compound of Formula IId:



Formula IId

wherein:

R_4 is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R_5 and R_6 are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

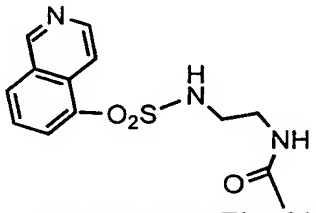
R_7 is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R_7 represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid.

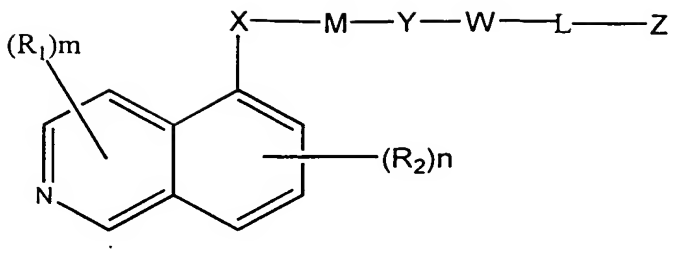
6. A compound of Formula III:



Arg-Pro-Arg-Thr-Glu-Ser-Phe

Formula III

7. A pharmaceutical composition comprising as an active ingredient a compound of Formula I:



Formula I

wherein:

R_1 and R_2 are independently selected from the group consisting of hydrogen, a lower alkyl group, a lower alkoxy group, substituted or unsubstituted phenyl group, a lower alkyl substituted with at least one substituent selected from the group consisting of a phenyl group, a halogen, hydroxyl, thiol, nitro, cyano, or amino group;

m and n are each independently 0-3;

X is selected from the group consisting of SO_2-NH, S and O;

M represents substituted or unsubstituted alkylene of 1-4 carbon atoms;

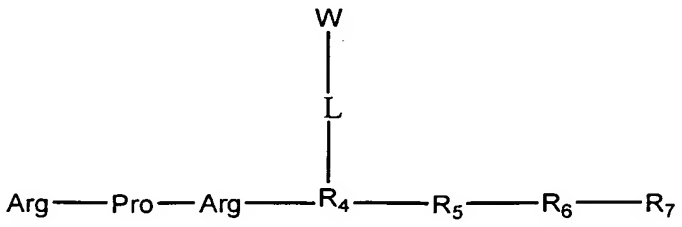
Y is selected from the group consisting of amide, amine, urea, carbamate, hydrazine or sulfonamide;

W is absent or is selected form the group consisting of substituted or unsubstituted alkylene, aliphatic, aromatic or heterocyclic moiety, of 1-18 carbon atoms;

L is absent or is selected from the group consisting of amide, amine, urea, carbamate, hydrazine or sulfonamide; and

Z is a peptide or peptidomimetic moiety of 4-12 residues in length capable of binding to the substrate site of PKB, and a pharmaceutically acceptable diluent or carrier.

8. A pharmaceutical composition comprising as an active ingredient the compound of the general Formula IIa:



Formula IIa

wherein:

R is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R and R are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

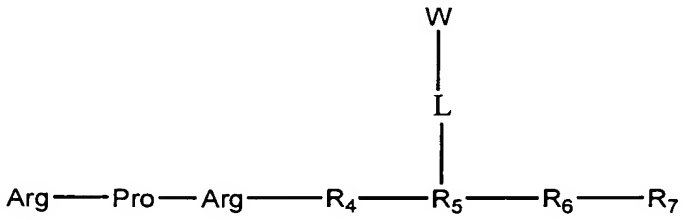
R is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid, and a pharmaceutically acceptable diluent or carrier.

9. A pharmaceutical composition comprising as an active ingredient the compound of the general Formula IIb:



Formula IIb

wherein:

R is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R and R are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

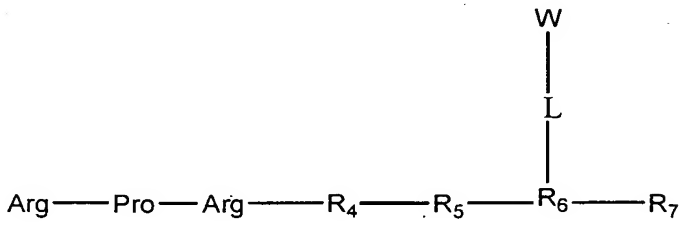
R is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid, and a pharmaceutically acceptable diluent or carrier.

10. A pharmaceutical composition comprising as an active ingredient the compound of the general Formula IIc:



Formula IIc

wherein:

R is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R and R are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

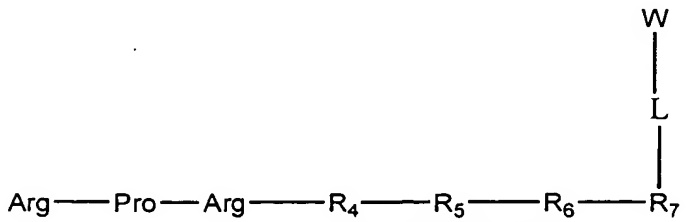
R is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid, and a pharmaceutically acceptable diluent or carrier.

11. A pharmaceutical composition comprising as an active ingredient the compound of the general Formula IId:



Formula IId

wherein:

R is selected from the group consisting of threonine, glutamic acid allyl ester, glutamic acid benzyl ester, homocitrulline, lysine, methionine, norleucine, ornithine, arginine, and alanine;

R and R are selected from the group consisting of Serine, Glutamic acid allyl ester, and alanine;

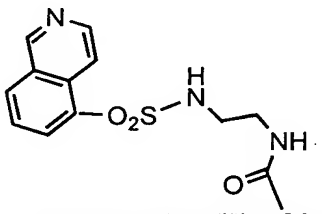
R is selected from the group consisting of phenylalanine, homoleucine, norleucine, glutamic acid allyl ester and glutamic acid benzyl ester;

the solid line connected R_1-R represents an amide bonds;

W is absent or is N-(8-sulfonamide-5-isoquinoline)ethylenediamine;

L may be absent or is selected from the group consisting of glycine, β-alanine, aminobutyric acid and aminopentanoic acid, and a pharmaceutically acceptable diluent or carrier.

12. A pharmaceutical composition comprising as an active ingredient a compound of Formula III:



Arg-Pro-Arg-Thr-Glu-Ser-Phe

Formula III

and a pharmaceutically acceptable diluent or carrier.

13. A pharmaceutical composition for inhibiting protein kinase comprising as an active ingredient a compound according to any one of claims 1-6, and a pharmaceutically acceptable diluent or carrier..

14. A method of treatment of a disease comprising administering to a patient in need thereof a pharmaceutical composition comprising as an active ingredient a therapeutically effective amount of a compound according to any one of claims 1-6.

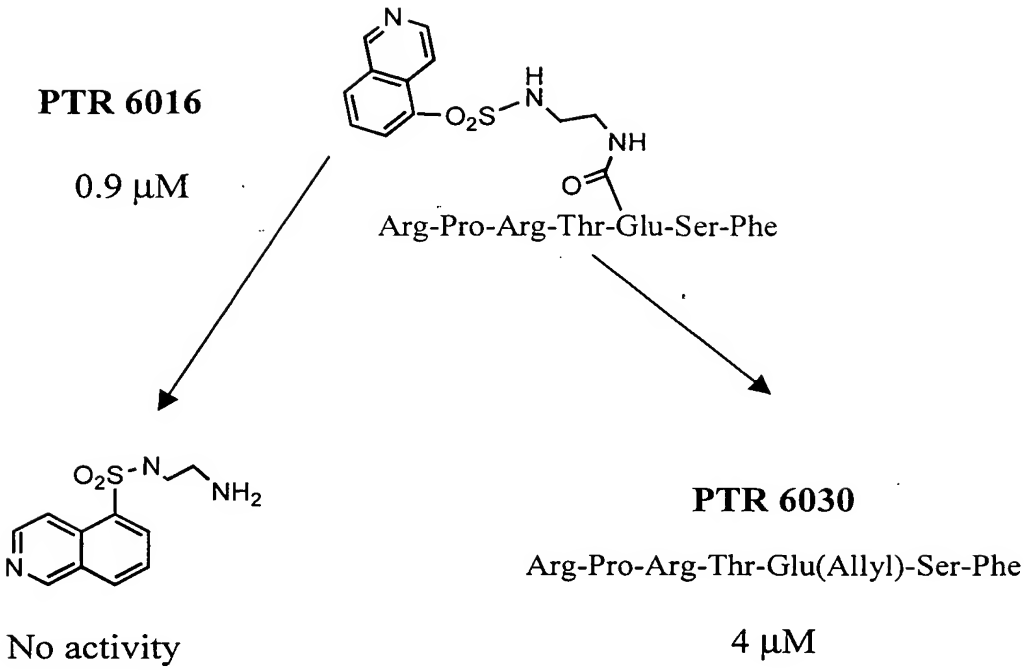
15. A method according to claim 14 wherein the disease is selected from the group comprising cancers, diabetes, cardiovascular pathologies, hemorrhagic shock, obesity, inflammatory diseases, diseases of the central nervous system, and autoimmune diseases.

16. A method of diagnosis of a disease comprising administering to a patient in need thereof a pharmaceutical composition comprising as an active ingredient a diagnostically effective amount of a compound according to any one of claims 1-6.

For the Applicants,

Cynthia A. Webb
Patent Attorney

Figure 1.


PTR 6016
0.9 μM
Arg-Pro-Arg-Thr-Glu-Ser-Phe
No activity
PTR 6030
Arg-Pro-Arg-Thr-Glu(Allyl)-Ser-Phe
4 μM

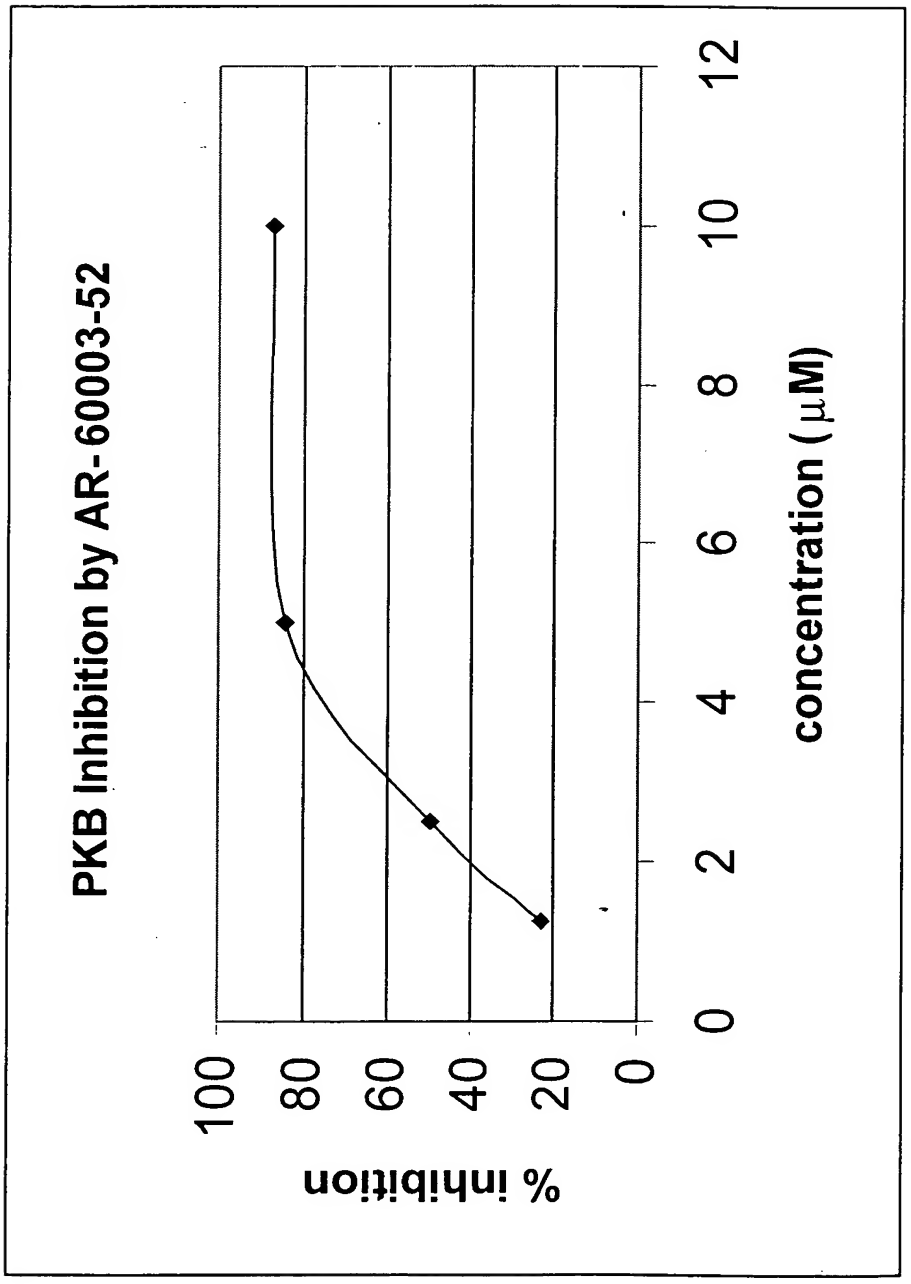

PKB Inhibition by AR-60003-52
% inhibition
100
80
60
40
20
0
0
2
4
6
8
10
12
concentration (μM)

Figure 2.